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EFFECTS OF SMALL-PARTICLE AEROSOLS OF RIMANTADINE AND RIBAVIRIN--ETC(U)
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The respiratory pathophysiology of A2 influenza infection was studied in mice treated with small-particle aerosols (SPA) of rimantadine or ribavirin. Untreated infections in mice resulted in survival rates of 15% or less, and were characterized by (a) severe hypoventilation (decreased P_{O_2} and increased P_{CO_2}), (b) compensated respiratory acidosis (increased P_{CO_2} and HCO_3^-, with normal pH), (c) pneumonia with increased ratio of wet/dry lung weight, and (d) hypothermia. Treatment with SPA of rimantadine (21 mg/kg/day for 4 days) beginning 72 h after virus challenge significantly improved survival rate (80%) but failed ~~(cont'd)~~

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to alter lung pathology found in infected, untreated mice. Rimantadine treatment decreased somewhat the severity of hypoventilation, respiratory acidosis, lung wet weight, hypothermia, and lung virus titers, observed in infected-untreated mice. SPA of ribavirin (26 mg/kg/day for 4 days) initiated 6 h after SPA exposure of mice to virus significantly improved survival rate (95%) and reduced lung virus titers and lung pathology. Gas exchange and pulmonary edema in ribavirin-treated, infected mice were significantly improved over those of infected, untreated controls. The mechanisms for increased survival rates induced by SPA of rimantadine remain uncertain, since increased survival rates could not be ascribed entirely to improvements in lung functions. In contrast, however, ribavirin treatment appeared to improve survival rates by reducing major lung pathology and pulmonary dysfunction. This was probably mediated through the antiviral effects of ribavirin.

Effects of Small-Particle Aerosols of Rimantadine and Ribavirin on Arterial Blood pH and Gas Tensions, and Lung Water Content of A2 Influenza-Infected Mice

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Running head: BLOOD GAS TENSIONS IN TREATED MOUSE INFLUENZA

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In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on the Revision of the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Research Council. The facilities are fully accredited by the American Association for Accreditation of Laboratory Animal Care.

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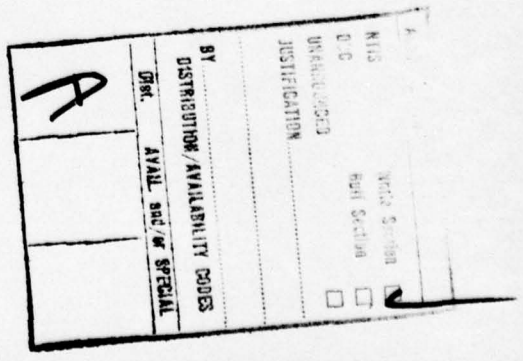
ABSTRACT

The respiratory pathophysiology of A2 influenza infection was studied in mice treated with small-particle aerosols (SPA) of rimantadine or ribavirin. Untreated infections in mice resulted in survival rates of 15% or less, and were characterized by (a) severe hypoventilation (decreased P_{O_2} and increased P_{CO_2}), (b) compensated respiratory acidosis (increased P_{CO_2} and HCO_3^-, with normal pH), (c) pneumonia with increased ratio of wet/dry lung weight, and (d) hypothermia. Treatment with SPA of rimantadine (21 mg/kg/day for 4 days) beginning 72 h after virus challenge significantly improved survival rate (80%) but failed to alter lung pathology found in infected, untreated mice. Rimantadine treatment decreased somewhat the severity of hypoventilation, respiratory acidosis, lung wet weight, hypothermia, and lung virus titers, observed in infected-untreated mice. SPA of ribavirin (26 mg/kg/day for 4 days) initiated 6 h after SPA exposure of mice to virus significantly improved survival rate (95%) and reduced lung virus titers and lung pathology. Gas exchange and pulmonary edema in ribavirin-treated, infected mice were significantly improved over those of infected, untreated controls. The mechanisms for increased survival rates induced by SPA of rimantadine remain uncertain, since increased survival rates could not be ascribed entirely to improvements in lung functions. In contrast, however, ribavirin treatment appeared to improve survival rates by reducing major lung pathology and pulmonary dysfunction. This was probably mediated through the antiviral effects of ribavirin.



Rimantadine hydrochloride (4,12,15) and ribavirin (4,15-17) have been reported to be effective against influenza A infections, both in vitro and in vivo. Small-particle aerosols (SPA) of rimantadine used to treat influenza A infections of mice have markedly improved percentage survival without decreasing lung lesions. Oral ribavirin therapy of influenza A and B infections of mice resulted in marked reductions in lung virus titers and lung lesions, and increased survival (5). Percentage survival of ribavirin-treated, influenza A-infected mice was increased when the drug was given by SPA beginning 6 h postinfection (16,17). In comparison to earlier treatment, survival was decreased when ribavirin therapy by either the intraperitoneal (i.p.) or aerosol route was delayed until 72 h postinfection (16,17).

This report describes arterial blood pH and gas tensions, and lung water contents of (a) A2 influenza-infected and noninfected mice, (b) infected mice treated with SPA of rimantadine, and (c) infected mice treated with SPA of ribavirin. Physiologic evidence is provided which suggests that rimantadine and ribavirin increase survival rates of infected-treated mice possibly by different mechanisms.

MATERIALS AND METHODS

Animals and procedures. Seven-week-old female mice, Tac:(SW) fBR, were used in all studies. At periodic intervals after infection, five to eight mice per group were selected at random and weighed. Mice were anesthetized in a chamber through which flowed 1% halothane in room air at a rate of 2 liters/min. Blood was collected from the anterior abdominal aorta into dry, heparinized syringes. Rectal temperatures of mice were recorded before anesthesia and again immediately prior to blood sampling; the latter was used for temperature correction of pH values. Percentage survival was calculated at 14 days from data on separate groups of mice, which were infected and treated simultaneously with all other experimental groups.

Virus. The mouse-adapted A/Aichi/2/68 (H_3N_2) strain of influenza virus was given to mice as an SPA with a mass median diameter of 2.2 μm, as previously described (15). Preparation of inocula, sampling and titration of the exposure cloud, lung virus titers, lung lesion scores, and histopathology were accomplished as previously reported (15-17). The exposure dose of virus in all experiments was between $10^{4.9}$ and $10^{5.6}$ egg median infective doses (EID_{50}), an approximate LD_{90}.

Drug. Rimantadine (α-methyl-1-adamantanemethylamine hydrochloride, E. I. DuPont de Nemours and Co., Inc., Newark, N.J.) and ribavirin (Nucleic Acid Research Institute of ICN Pharmaceuticals, Inc., Irvine, Calif.) were dissolved in sterile, triple-distilled water and given as SPA, as previously described (15-17). Presented doses were 21 and 26 mg/kg/day for rimantadine and ribavirin, respectively.

Blood gas tension. Arterial blood samples were analyzed within 10 min of collection for partial pressure of oxygen (P_{O_2}) and of carbon

dioxide (P_{CO_2}) and pH. All determinations were performed using an automated analyzer (Model 165, Corning Instruments, Inc., Medfield, Mass.), calibrated at 37 C. Algorithms of Ruiz et al. (14) were used to correct pH values to body temperature and to calculate bicarbonate.

Lung water content. Immediately after blood sampling, the thorax was opened and all lung tissue was removed, weighed, and dried to constant weight in a vacuum oven. The ratio of wet/dry lung weight was calculated.

Rimantadine studies. Approximately 1300 mice were used in duplicate experiments. Mice were allocated at random into one of three groups: uninfected-untreated (UU), infected-untreated (IU), and infected-treated (IT). Rimantadine therapy was initiated 72 h after infection and given as a continuous SPA 22 h/day for 4 days (16,17). Three mice were selected at random on day 7 to verify lung virus titers and lung lesion scores.

Ribavirin studies. Approximately 350 mice for each of two experiments were allocated at random into one of three groups, as for the rimantadine studies. Therapy was initiated at 6 and 72 h postinfection in the two respective experiments. Drug was dministered as an SPA for 80 min at the same time of day on 4 consecutive days (16,17). Lung virus titers were determined and lung lesions were scored on day 7. In both experiments, IU mice were sham-treated with distilled water (drug vehicle).

Drug controls. As a separate experiment, three groups of 40 uninfected mice each were given SPA of rimantadine (22 h/day for 4 days), ribavirin (80 min/day for 4 days), or distilled water (80 min/day for 4 days) as an SPA.

Statistics. Treatment groups were compared using one-way analysis of variance. Differences were considered significant when $P \leq 0.05$.

Data from replicate experiments were pooled after it was determined by a two-way analysis of variance that there were no marked differences between experiments.

RESULTS

Influenza treated with rimantadine. Survival was 80 vs. 7% in the respective IT and IU groups while lung virus titers were approximately 1 $\log_{10}$ lower in the former than in the latter (Fig. 1A). All deaths occurred between 4 and 9 days postinfection in both groups of mice, with the majority between days 6 and 8 (Fig. 1A). No significant differences were observed between IU and IT mice in lung lesion scores (Fig. 1A).

Arterial P_{O_2} values decreased in both IU and IT mice and reached minimum values on days 8 and 6, respectively (Fig. 2A). Subsequently, P_{O_2} values increased in surviving mice from both groups; however, between 7 and 14 days postinfection values for IT mice increased earlier, and were significantly closer to normal base-line values than P_{O_2} values of IU mice (Fig. 2A). P_{CO_2} values increased in both IU and IT mice in parallel with the decrease in P_{O_2} (Fig. 2B). The P_{CO_2} values for IT mice were significantly closer to base-line values than values for IU mice on days 5, 6, 10, and 12.

Blood pH values were not significantly different among UU, IU, and IT mice except for day 12 postinfection when IU mice were significantly more acidotic than IT mice (Fig. 2C). Blood bicarbonate values were maximum in IU and IT mice on day 5 or 6 postinfection and subsequently decreased to base-line values (Fig. 2D). Rectal temperatures decreased through day 6 in IU and IT mice; however, the magnitude of the depression was significantly less in IT mice. Rectal temperature in both IU and IT mice was close to base-line values by day 19 (Fig. 2E). Wet/dry lung weight ratios increased after infection in both IU and IT mice and reached respective maximum values on days 7 and 6 (Fig. 2F). Subsequently, in recovering mice, lung water content decreased in both groups, but remained

elevated above values of UU controls (Fig. 2F). Body weights of both IU and IT mice decreased through day 6. This weight loss continued through day 8 in IU mice, but not in IT mice. The latter were significantly heavier than IU mice by day 7 postinfection (Fig. 3A). Rimantadine treatment of uninfected control mice caused no significant changes in P_{O_2}, P_{CO_2}, pH, HCO_3^-, rectal temperature, lung water content, or body weight when compared to values for UU mice.

Influenza treated with ribavirin. Ribavirin therapy initiated at 6 h postinfection resulted in lower lung lesion scores and lung virus titers when compared to IU mice (Fig. 1B). Survival rates were 5 and 95% in IU and IT mice, respectively (Fig. 1B). By contrast, ribavirin therapy initiated at 72 h postinfection resulted in no significant change in lung lesion scores or lung virus titers (Fig. 1C). When ribavirin therapy was initiated at 72 h, survival rates were 15% in IT mice, and 10% in IU mice (Fig. 1C).

A2 influenza infection in mice without subsequent ribavirin treatment (IU) in this series of experiments showed no significant differences in measured values for IU mice in the previous rimantadine experiments (Figs. 2, 4, and 5). Ribavirin therapy, however, initiated at 6 h postinfection (IT) produced significant changes when compared with physiologic values in IU mice (Fig. 4A-F). Arterial P_{O_2} and P_{CO_2} values in IT mice were significantly closer to base-line values when compared to IU mice throughout most of the experimental period (Fig. 4A, B). Arterial blood pH values for IU and IT mice were significantly different on day 8 postinfection when IT mice developed an acidosis (Fig. 4C). Blood bicarbonate values were unchanged from normal base-line values in IT mice in contrast to their significant elevation in IU mice by day 5

(Fig. 4D). Rectal temperatures of IT mice remained normal, while IU mice exhibited significant hypothermia throughout the experimental period (Fig. 4C). Ribavirin treatment initiated 6 h postinfection markedly diminished the pneumonia found in IU mice (Fig. 4F). Infected, ribavirin-treated (IT) mice showed significantly less weight loss compared with IU mice by day 7 and weighed more than UU mice by day 12 postinfection (Fig. 3B).

When ribavirin therapy was initiated 72 h postinfection, the effectiveness of treatment was markedly diminished (Fig. 5A-F) when compared to treatment initiated at 6 h postinfection. In general, P_{O_2} and P_{CO_2} values of recovering IT mice still returned to normal base-line values significantly faster than values in IU mice (Fig. 5A, B). Arterial blood pH and HCO_3^- values of IT and IU mice were not different throughout the experimental period (Fig. 5C, D). Significantly less severe hypothermia (Fig. 5E) and lung wet weight (Fig. 5F) were noted in IT recovering mice than in IU mice. Both IU and IT mice exhibited significant weight loss as compared with UU mice during the experimental period (Fig. 3C). Treatment of uninfected control mice with ribavirin caused no significant changes in P_{O_2}, P_{CO_2}, pH, HCO_3^-, rectal temperature, or lung water contents when compared to values for UU mice.

DISCUSSION

Experimental A2 influenza in the laboratory mouse is characterized by (a) severe hypoxemia (decreased P_{O_2}), (b) inadequate alveolar ventilation (increased P_{CO_2}), (c) compensated respiratory acidosis (normal blood pH and increased P_{CO_2}, and HCO_3^-, (d) increased lung wet

weight, (e) hypothermia, (f) weight loss, and (g) high mortality 5-9 days postinfection. Significant physiologic changes in infected-untreated mice generally occur several days subsequent to peak lung virus titers and lung lesions (9,15). Data accumulated on percentage survival, lung virus titers, and lung lesions confirm the high morbidity and mortality caused by this disease in the laboratory mouse (15-17). Mortality in groups of infected mice treated at 72 h postinfection with SPA of ribavirin was higher than reported previously (16,17). This difference could be an artifact due to the small number of mice (20/group) set aside for determination of survival, a defect in the dissemination of ribavirin to treated mice, or a difference in animal susceptibility to the virus.

Ribavirin therapy initiated 6 h postinfection prevented the pathophysiologic changes seen in infected-untreated mice and significantly increased percentage survival. When ribavirin therapy was delayed until 72 h postinfection, no significant improvement in pulmonary function was observed until late in the course of the infection, and mortality remained elevated. It has been reported that peak lung virus titers are reached 48 h postinfection (9) and that by 72 h, bronchopneumonia is well established (16,17). Therefore, the prevention of severe pathophysiologic change in the early treated group can reasonably be attributed to the antiviral properties of ribavirin (16). When ribavirin therapy was delayed until after development of bronchopneumonia and peak lung virus titers, the drug was clearly less effective in preventing pulmonary dysfunction and mortality.

When SPA of rimantadine were administered 72 h postinfection, less severe pathophysiologic changes were observed late in the course of

infection. The rimantadine-treated mice recovered earlier than infected-untreated controls and had significantly improved survival rates. The slight but significant changes in P_{O_2}, P_{CO_2}, rectal temperature, and lung wet weight beginning 6 or 7 days postinfection may be very important in improving survival rates and speeding recovery of the infected, rimantadine-treated host. This is particularly noteworthy in view of the absence of any detectable lessening of lung lesions and only a slight reduction in lung virus titers of treated mice. A reduction greater than 1 $\log_{10}$ in lung virus titers was also observed by Stephen, et al. (15) in rimantadine-treated mice which may be all that is necessary to improve survival rates significantly. It should also be noted that a direct comparison of the results with rimantadine to those of ribavirin was avoided in this study because presented dosages of both drugs and treatment schedules (22 h/day with the former vs. 80 min/day with the latter) were not identical for the two compounds.

In infected-untreated mice, P_{CO_2} increased at the same time as hypoxemia developed (Fig. 2A, B). This type of response is characteristic of either severe, restrictive lung disease or depression of the carbon dioxide-sensitive chemoreceptors in the brain (2). In man, influenza infections are associated with restrictive lung disease, with hypercapnia occurring after a period of hypoxia (3). However, central nervous system (CNS) involvement in primary influenzal pneumonia has been reported (11). When mice are inoculated intracerebrally with non-neuroadapted type A influenza virus, CNS-type death occurs 5-7 days later (13). Treatment of type A influenza infection of mice with 85 or 100% oxygen decreases survival (1). In man, oxygen therapy of influenza infection may result in reduced ventilatory drive (6). This

finding in man and increased deaths of mice (1) suggest that the hypoxic stimulus to ventilation via the carotid and aortic chemoreceptors may assume major importance, possibly due to impaired function of the carbon dioxide-sensitive chemoreceptors and associated mechanisms in the CNS.

Both rimantadine hydrochloride and its structural analog amantadine hydrochloride increase survival rates of influenza-infected mice (5,7,12, 17). However, the efficacy of both drugs is limited to type A influenza virus infection (7,17). Treatment of natural influenza A infection in man with amantadine is associated with a shortened course of clinical illness and a more rapid resolution of the small airway dysfunction, with no effect on virus shedding or development of antibody titer (7,10). In addition, amantadine is an effective agent for the treatment of functional disturbance of the CNS, such as occurs in patients with Parkinson's disease, and has been shown to have an effect on central thermoregulatory control mechanisms of mice (8,10). If we assume that rimantadine produces central effects similar to amantadine, then drug effects on the CNS may also partially explain improved survival rates in groups of rimantadine-treated, A2 influenza-infected mice. Further study will be necessary to implicate CNS function as an important facet of successful influenza therapy.

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FIGURE LEGENDS

FIG. 1. Effect of small-particle aerosols of rimantadine or ribavirin on percentage survival, lung virus titers (LVT), and lung lesion score (LLS) of A2 influenza-infected mice. Rimantadine was administered for 4 days beginning 72 h postinfection. Ribavirin was administered for 4 days beginning at either 6 or 72 h postinfection. (Δ) Uninfected-untreated (UU); (O) infected-untreated (IU); (●) infected-treated (IT).

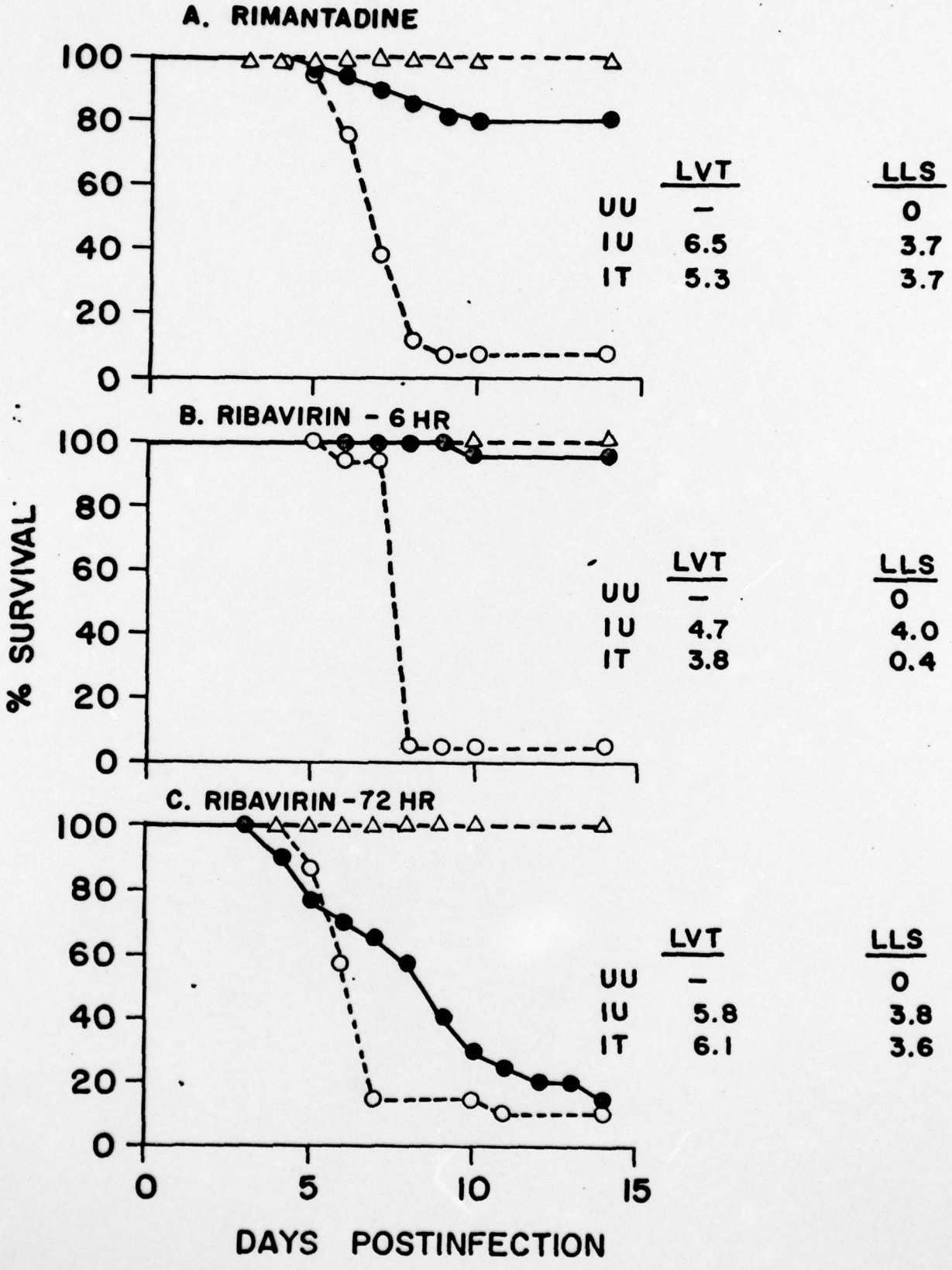
FIG. 2. Effect of small-particle aerosols of rimantadine initiated at 72 h postinfection for 4 days on arterial P_{O_2} and P_{CO_2}, pH, bicarbonate, rectal temperature, and wet/dry lung weight ratios of A2 influenza-infected mice. Significant differences ($P < 0.05$) between uninfected-untreated (UU, Δ) and infected-untreated (IU, O) mice are indicated by a bar (⊢⊣), and between infected-untreated (IU) and infected-treated (IT, ●) mice by asterisks (*).

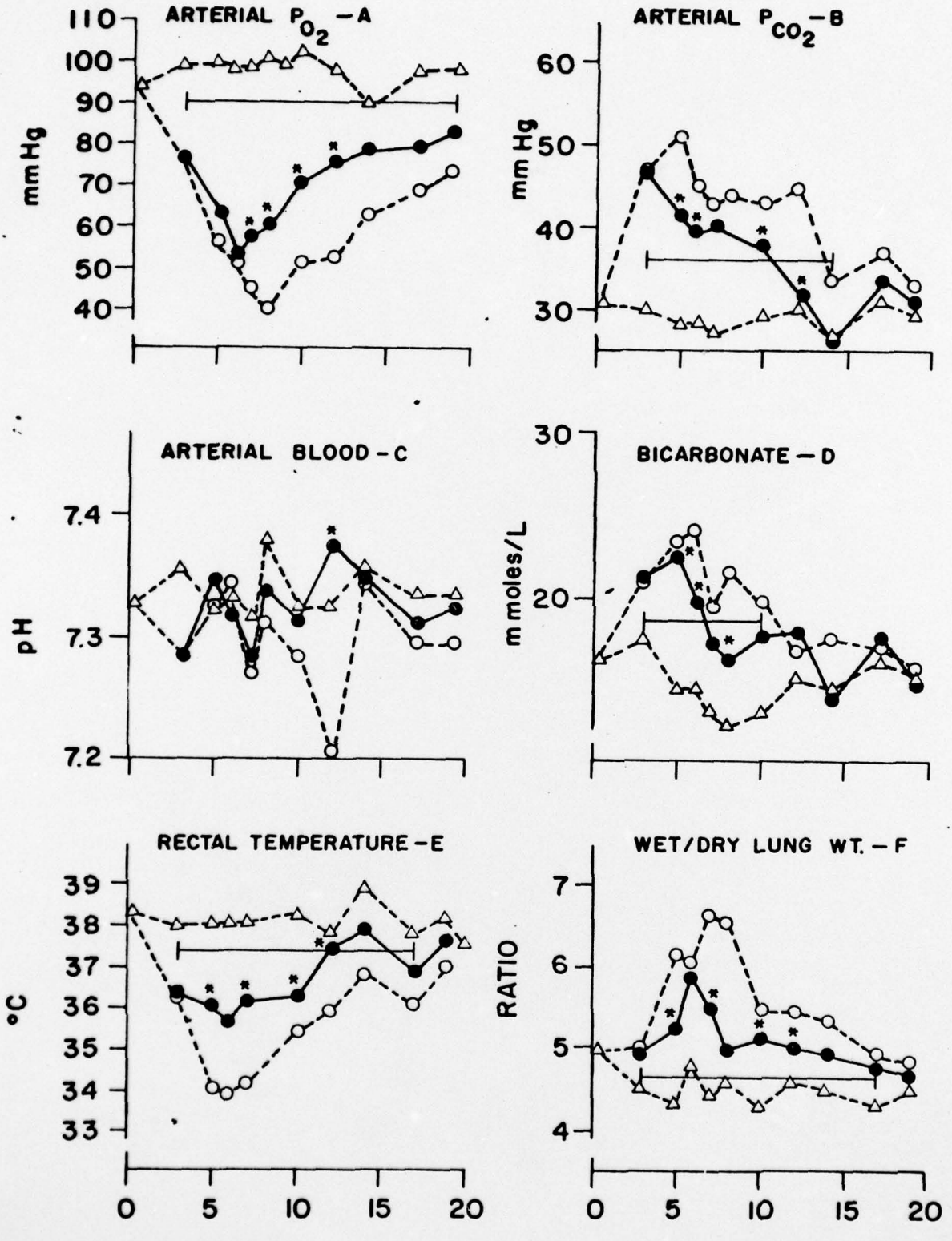
FIG. 3. Effect of small-particle aerosols of rimantadine or ribavirin on body weight of A2 influenza-infected mice. Rimantadine was administered beginning 72 h postinfection for 4 days, whereas ribavirin was administered beginning either at 6 or 72 h postinfection for 4 days. Results are presented as change in body weight, in grams, from mean values for day 0. Significant differences ($P \leq 0.05$) between uninfected-untreated (UU, Δ) and infected-untreated (IU, O) mice are indicated by a bar (⊢⊣), and between infected-untreated and infected-treated (IT, ●) mice by asterisks (*).

FIG. 4. Effect of small-particle aerosols of ribavirin on A2 influenza-infected mice with treatment initiated 6 h postinfection for 4 consecutive days as measured by arterial P_{O_2} and P_{CO_2}, pH, bicarbonate, rectal temperature, and wet/dry lung weight ratios. Significant differences

($P \leq 0.05$) between uninfected-untreated (UU, △) and infected-untreated (IU, ○) mice are shown with a bar (⊢⊣), and differences between infected-untreated (IU) and infected-treated (IT, ●) mice are shown by asterisks (*).

FIG. 5. Effect of small-particle aerosols of ribavirin on A2 influenza-infected mice with treatment initiated 72 h postinfection for 4 consecutive days as measured by arterial P_{O_2} and P_{CO_2}, pH, bicarbonate, rectal temperature, and wet/dry lung weight ratios. Significant differences ($P \leq 0.05$) between uninfected-untreated (UU, △) and infected-untreated (IU, ○) mice are shown with a bar (⊢⊣), and differences between infected-untreated (IU) and infected-treated (IT, ●) mice are shown by asterisks (*).


A. RIMANTADINE
B. RIBAVIRIN - 6 HR
C. RIBAVIRIN - 72 HR
% SURVIVAL
100
80
60
40
20
0
0
5
10
15
DAYS POSTINFECTION
LVT
LLS
UU – 0
IU 6.5 3.7
IT 5.3 3.7
UU – 0
IU 4.7 4.0
IT 3.8 0.4
UU – 0
IU 5.8 3.8
IT 6.1 3.6


ARTERIAL P_{O_2} – A
mm Hg
110
100
90
80
70
60
50
40
ARTERIAL P_{CO_2} – B
mm Hg
60
50
40
30
ARTERIAL BLOOD – C
pH
7.4
7.3
7.2
BICARBONATE – D
m moles/L
30
20
RECTAL TEMPERATURE – E
°C
39
38
37
36
35
34
33
WET/DRY LUNG WT. – F
RATIO
7
6
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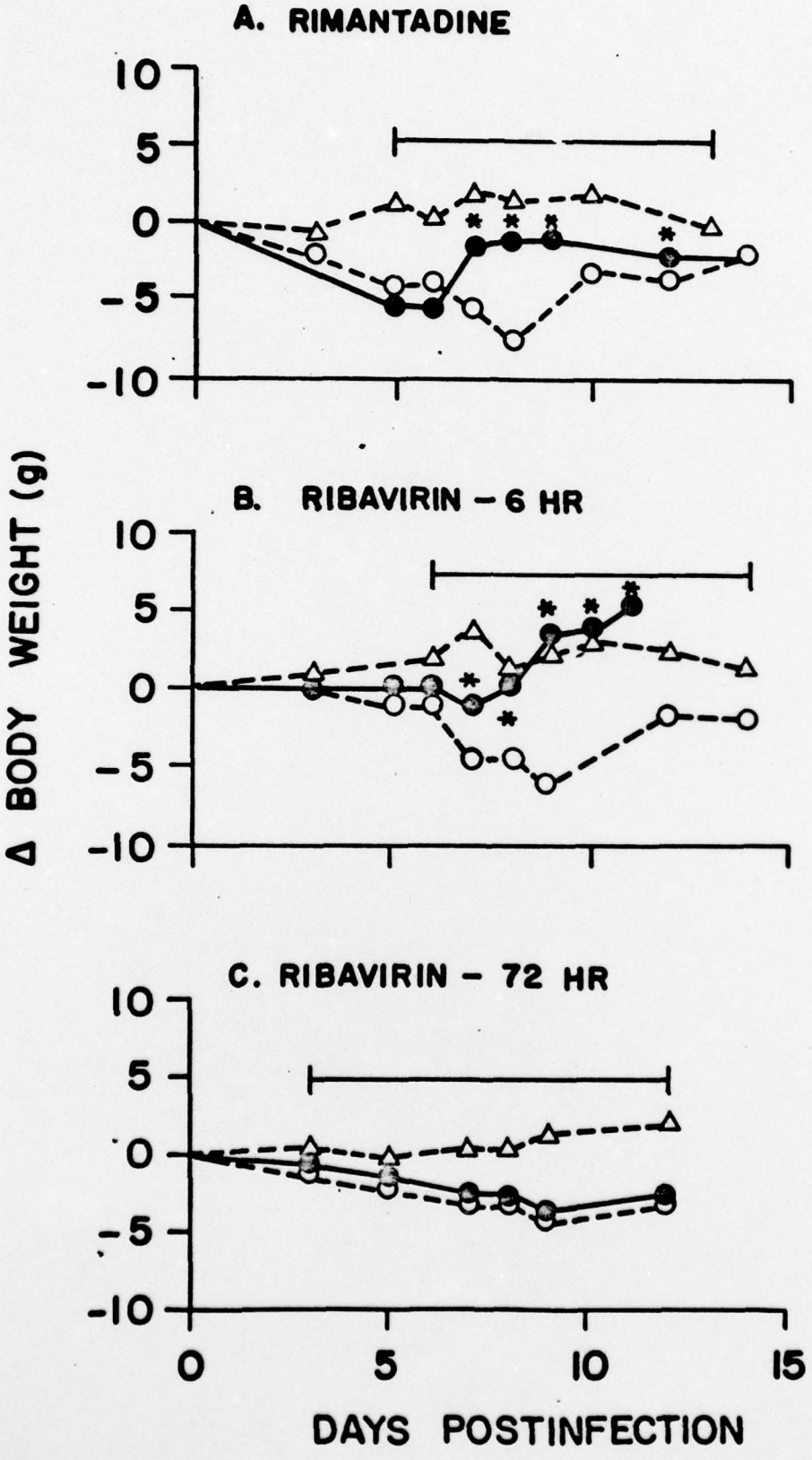
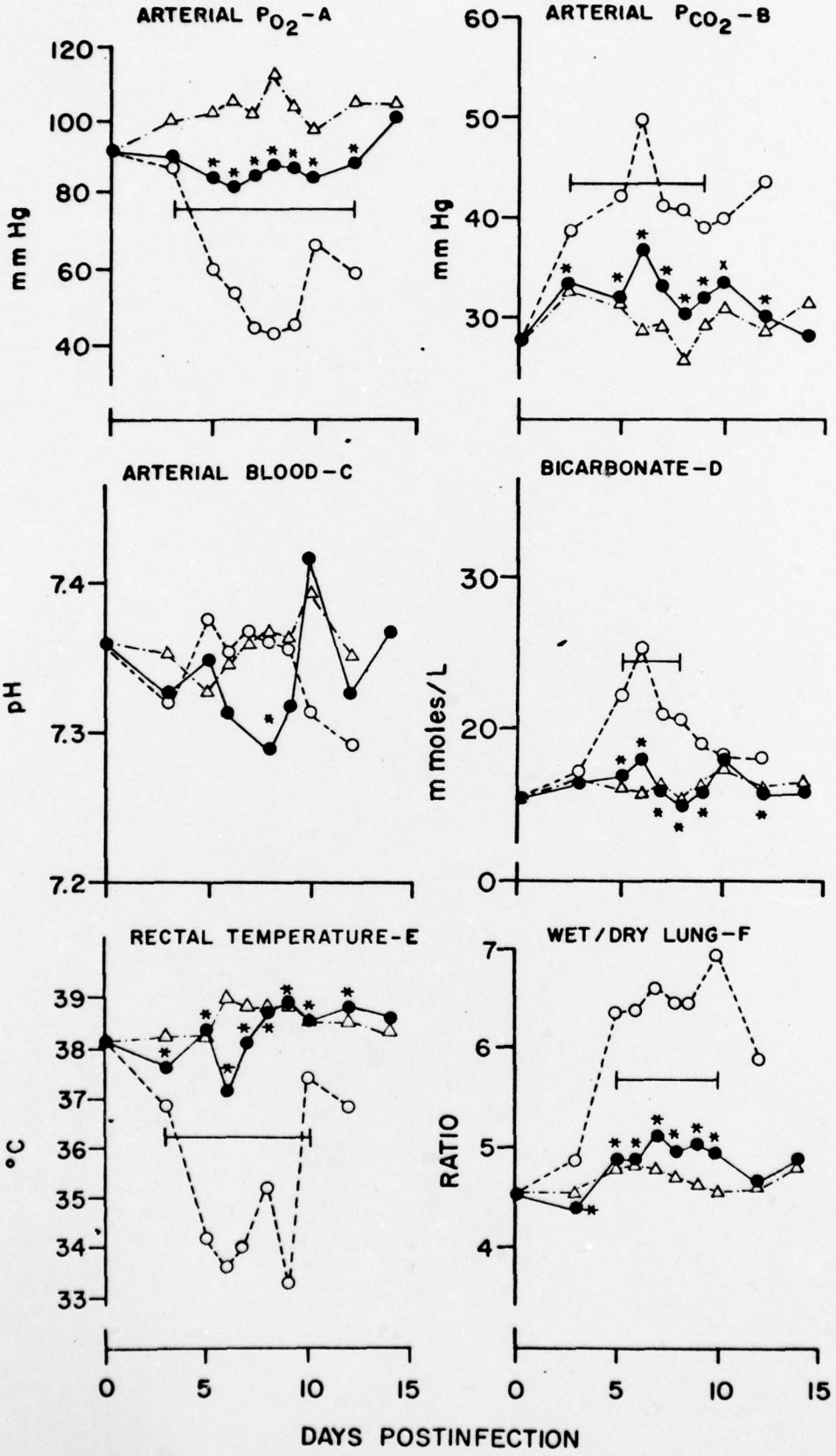
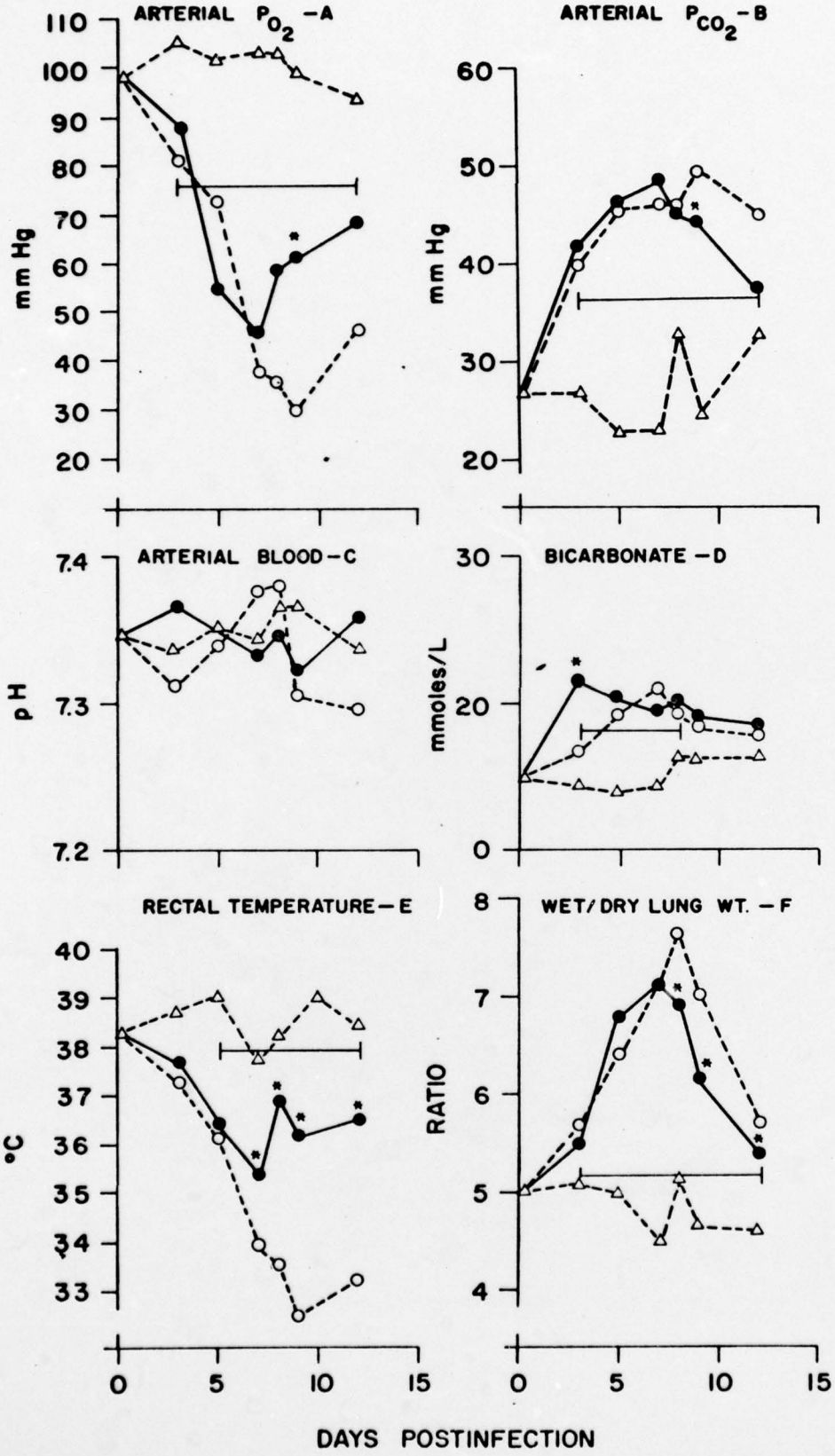
10
15
20
DAYS POSTINFECTION


A. RIMANTADINE
B. RIBAVIRIN – 6 HR
C. RIBAVIRIN – 72 HR
Δ BODY WEIGHT (g)
10
5
0
-5
-10
0
5
10
15
DAYS POSTINFECTION


ARTERIAL P_{O_2} - A
mm Hg
120
100
80
60
40
ARTERIAL P_{CO_2} - B
mm Hg
60
50
40
30
ARTERIAL BLOOD - C
pH
7.4
7.3
7.2
BICARBONATE - D
m moles/L
30
20
0
RECTAL TEMPERATURE - E
°C
39
38
37
36
35
34
33
WET / DRY LUNG - F
RATIO
7
6
5
4
0
5
10
15
DAYS POSTINFECTION


ARTERIAL P_{O_2} – A
ARTERIAL P_{CO_2} – B
ARTERIAL BLOOD – C
BICARBONATE – D
RECTAL TEMPERATURE – E
WET/DRY LUNG WT. – F
mm Hg
mm Hg
pH
mmoles/L
°C
RATIO
DAYS POSTINFECTION